STANFORD UNIVERSITY LIBRARY
DEC 27 1927

An Investigation of the Distribution of Nitrogen in Wool and Casein, When Treated in the Cold with Various Concentrations of Hydrochloric Acid

BY HENRY INGHAM STUBBLEFIELD

A Thesis

PRESENTED TO THE FACULTY OF
THE GRADUATE COLLEGE IN THE UNIVERSITY OF NEBRASKA
IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
DEPARTMENT OF CHEMISTRY

3781
N365

Lincoln, Nebraska
August 20, 1926

UNCLASSED
Dissertation
STANFORD UNIVERSITY LIBRARIES
WITHDRAWN

An Investigation of the Distribution of Nitrogen in Wool and Casein, When Treated in the Cold with Various Concentrations of Hydrochloric Acid

BY HENRY INGHAM STUBBLEFIELD

A Thesis

PRESENTED TO THE FACULTY OF
THE GRADUATE COLLEGE IN THE UNIVERSITY OF NEBRASKA
IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
DEPARTMENT OF CHEMISTRY

Lincoln, Nebraska
August 20, 1926

The writer wishes to express his sincere appreciation for the advice and ever helpful criticism of Dr. T. J. Thompson, under whose direction this work was carried out.

An Investigation of the Distribution of Nitrogen in Wool and Casein, When Treated in the Cold with with Various Concentrations of Hydrochloric Acid

INTRODUCTION

In so far as can be learned a quantitative study of the effect of hydrochloric acid of varying concentrations on protein material at ordinary room temperature has never been reported.

Fischer and Abderhalden,[1] et. seq., prepared several dipeptids by hydrolysis of silk fiber with concentrated sulfuric acid in the cold. Thompson-Merrill [2] studied the effect of various high concentrations of hydrochloric acid, and the time of boiling on the yield of cystine, prepared from wool. Hugouneq and Morel [3] studied the effect of high concentrations of hydrofluoric acid and the time of boiling on the hydrolysis of proteins. Denis [4] studied the effect of time of boiling with high concentrations of hydrochloric acid on the amount of amide nitrogen yielded by proteins. The above researches are typical of those made in this branch of protein chemistry.

It seemed worth while to study the effect of various concentrations of acid at room temperature on protein material. With this in view it was decided to determine the nitrogen distribution according to the method of Hausmann,[5] as modified by Osborne and Harris.[6]

In brief, the method of Hausmann as modified by Osborne and Harris is as follows: The protein to be studied is hydrolysed by boiling for several hours with several times its volume of concentrated hydrochloric acid, until hydrolysis is complete as indicated by a negative biuret test. The hydrolysate is then subjected to distillation in vacuo at 40°, 20 mm. pressure, and evaporated almost to dryness. This operation removes nearly all of the hydrochloric acid, and the distillate is discarded. The residue is diluted to about three hundred cubic centimeters with ammonia-free distilled water, and rendered faintly but distinctly alkaline with magnesium cream, which has been boiled to free it from ammonia. The mixture is then distilled in vacuo, the distillate being collected

[1] Ber. 39, 752, (1906), et. seq.
[2] J.A.C.S. 43, 2688, (1921).
[3] Comp. Rend. 146, 11291, (1908).
[4] J. Biol. Chem. 8, 427, (1910).
[5] Zeit. physiol. Chem. 29, 92, (1899).
[6] J.A.C.S. 25, 323, (1903).

in excess of acid. The ammonia is determined by titration against standard alkali, methyl-red being used as the indicator, which is added directly to the acid in the receiver. This gives the amide nitrogen.

The residue in the distillation flask is then filtered thru a nitrogen-free filter and washed carefully with ammonia-free water. The precipitate from the ammonia distillation together with the filter paper is tested for nitrogen by the Kjeldahl method. The nitrogen thus determined is the humin nitrogen.

The filtrate and washings from the ammonia determination are acidulated with 5 gm. (3 cc.) concentrated sulfuric acid, and treated with a solution of 20 per cent phosphotungstic acid in 5 per cent sulfuric acid. This precipitates the diamino acids as their phosphotungstates. The reaction mixture is allowed to stand 24 hours, when it is filtered. The precipitate is washed three times with a 5 per cent solution of phosphotungstic acid in 5 per cent sulfuric acid. The precipitate and filter are transferred to a Kjeldahl flask and the nitrogen determined by that method. This gives the diamino nitrogen.

The filtrate and washings from the diamino determination contain the nitrogen in the monoamino acids. This may be diluted to volume and an aliquot portion subjected to a Kjeldahl determination.

A number of objections have been raised to the accuracy of Hausmann's method; Henderson [7] held that the amount of amide nitrogen was variable, depending on the strength of acid and the time of boiling. Schultze and Winterstein [8] concluded that certain of the monoamino acids were precipitated by the phosphotungstic acid. Kutscher,[9] Hart,[10] Wetzel,[11] and Chittenden and Eustis,[12] studied the phosphotungstic acid precipitation in minute detail. Their conclusions were that certain monoamino acids are precipitated by the phosphotungstic acid treatment (e.g. phenylalanine), and that the precipitation of the diamino acids is not complete.

Osborne and Harris, loc. cit. tested the method with extreme care, as did Gumbel,[13] and Rothera.[14] They concluded that the objection of Henderson with regard to the variability of the amide nitrogen determination was unwarranted,

[7] Zeit. physiol. chem. 29, 47, (1900).
[8] ibid, 33, 563, (1901).
[9] ibid, 31, 315, (1901).
[10] ibid, 33, 347, (1901).
[11] ibid, 29, 403, (1901).
[12] Am. J. Physiol. 3, 31, (1903).
[13] Beitr. Chem. Physiol. Path. 5, 297, (1904).
[14] ibid, 5, 442, (1904).

and that the errors in incomplete precipitation of diamino acids and the precipitation of monoamino acids in the phosphotungstic acid precipitation practically compensate each other. Their work leads to the conclusion that if uniform conditions are maintained that the method is decidedly valuable for comparative data. However, it is not to be recommended for quantitative determinations where similarity of conditions cannot be maintained.

The present work is an investigation as to the effect of various concentrations of hydrochloric acid on the distribution of nitrogen in casein and wool, when allowed to stand at room temperature, as compared with the distribution of nitrogen when the substances are boiled under a reflux with concentrated hydrochloric acid.

In addition to the above study which is herein reported, investigations as to the nature of the products of the hydrolysis are to be subsequently undertaken, particularly with regard to the types of compounds which can be isolated. It is hoped that it will be possible to isolate and identify polypeptids from the hydrolysates, Fischer,[15] et seq.

In view of this intended study it was decided to use larger quantities of the casein and wool than is the usual case, altho the use of small quantities (1 gm. or less) is rendered necessary in the studies reported in the literature by Osborne and others, because of the rarity of the substances and the difficulty encountered in their isolation.

At the beginning of the present work a preliminary study of the Osborne-Harris modification of Hausmann's method was carried out with a view to testing its applicability.

1. To ascertain whether or not the diamino and monoamino determinations were complete, a check on results was made as follows: The solutions after removal of the humin precipitate were diluted to known volume and total nitrogen determined by Kjeldahl, the mono and diamino values were then run as usual. The values when added were found to check with the value found for the nitrogen in the two groups together.

2. Osborne and Harris recommend the concentration in vacuo of the filtrate and washings from the humin precipitate. Since this involves the additional vacuum distillation it seemed worth while to eliminate the step if practicable. Therefore, determinations were made wherein the filtrates and washings from the humin precipitate were evaporated in

[15] Ber. 36, 2982, (1903).

vacuo and then diluted to the same volume each time before the addition of the phosphotungstic acid precipitant. The operation was repeated, omitting the vacuum concentration and the filtrate and washings diluted to the same volume each time. Upon comparison of results in the two sets of conditions, no appreciable variance in the monoamino or diamino values were noted. Consequently, the omission of the vacuum concentration seemed justifiable.

3. The advisability of determining the monoamino nitrogen directly, rather than by loss as did Osborne and Harris, was considered. If the entire filtrate from the diamino precipitate is digested and nitrogen determined by Kjeldahl, " bumping " in the process of digestion is so excessive that the procedure is rendered impossible. The plan of diluting the filtrate and washings to a known volume and then determining the nitrogen in an aliquot part by Kjeldahl was adopted. The results were in sufficient agreement to justify their consideration.

4. The advisability of using perchloric acid (60 per cent) in the digestion of the Kjeldahl determinations was considered. Test runs were made, using varying quantities of perchloric acid, ranging from 3 cc. to 15 cc., with the hope that the digestion would be speeded up. The idea was discarded, because it was found that when sufficient quantity of perchloric acid was used to materially lessen the time for digestion, that a loss of nitrogen occurred which was too great to justify the continuance of the procedure.

Subsequent to the above noted studies and modifications the technique as described in the following experimental discussion was adopted.

EXPERIMENTAL PART

One hundred gram samples of clean wool, purchased locally in the form known as " wool batting " were washed in ether to remove any residual wool fat. This was placed in three liter flasks, and two liters of hydrochloric acid added. The strengths of the various acid solutions ranged from concentrated (sp. gr. 1.18-1.19, approximately 12N to one-tenth normal as tabulated below in Table I. The flasks were closed with tightly fitting rubber stoppers, and frequently shaken. Biuret tests were made from time to time to ascertain the point of apparent completeness of hydrolysis. The samples of casein (Pfanstiehl's grade known as " highest purity ") were prepared in a similar manner.

It was found that only the most concentrated acid affected

complete solution of the wool and casein, there being considerable suspended material in the bottom of the flasks. This substance when filtered was found to give a negative biuret test. In order to obtain uniform samples of the hydrolysates a special pipette was used having an opening of 2 mm. diameter, and calibrated to deliver 20 cc.; 20 cc. of the solution corresponding to one gram of the original solid material.

The analyses were carried out as follows:

Upon the completion of hydrolysis as indicated by the negative biuret test, a 20 cc. sample was removed from the rapidly agitated solution. This was transferred to a 1.5 liter round-bottom short-necked flask, fitted with a two-holed rubber stopper, containing a safety still-head and a glass tube. This tube was drawn out to a fine opening which extended down into the liquid, the upper opening being fitted with a rubber tube closed by a screw clamp to regulate a stream of air into the liquid. The solution was diluted to two hundred cubic centimeters with ammonia-free distilled water. It was then subjected to distillation in vacuo, the pressure being maintained at about 20 mm. and the temperature not being allowed to run above 80° C. The solution was evaporated to one or two cubic centimeters in volume. This process removes nearly all of the hydrochloric acid.

1. The residue was then diluted to three hundred cubic centimeters with ammonia-free distilled water. A solution of magnesium oxide was added until the reaction was faintly but distinctly alkaline to litmus, the magnesia cream having been boiled to remove any ammonia that might be present. After addition of the magnesia the solution was subjected to distillation in vacuo (20 mm. pressure, temperature being kept below 40° C.), the received being a 300 cc. capacity distilling flask, containing 10 cc. of N/14 HCl. The side tube of the distilling flask was fitted with 50 cc. distilling flask containing 5 cc. of the standard acid. The side tube of the first flask projecting under the surface of the liquid in the smaller distilling flask, which acted as a safety trap to catch any of the ammonia which might not be retained in the acid in the larger distilling flask. The indicator, methyl-red, was added directly to the acid in the receivers. About three-fourths of the solution was distilled over, and titrated against N/14 NaOH. The number of cubic centimeters of acid neutralized by the ammonia being equivalent to milligrams of nitrogen, as amide nitrogen.

2. The residue remaining in the reaction flask after the ammonia had been distilled off contains the humin, diamino, and monoamino nitrogen. This residue was washed onto a nitrogen-free filter and washed five times with ammonia-free water, and the filter paper, with the precipitate subjected to nitrogen determination by the Kjeldahl method. This is the humin nitrogen.

3. The filtrate and washings from the humin determination containing monoamino and diamino nitrogen are treated with 5 gm. (3 cc.) concentrated sulfuric acid, and 30 cc. of a solution of 20 per cent phosphotungstic acid in 5 per cent sulfuric acid. The diamino acids are precipitated as their phosphotungstates. The solutions were allowed to stand for forty-eight hours to assure complete precipitation. They were then filtered and the precipitates washed with a 2.5 per cent solution of phosphotungstic acid in 5 per cent sulfuric acid, 200 cc. of the wash solution being used, and added in three equal portions, each portion being allowed to drain completely before the addition of the next portion. The precipitate and filter paper were subjected to a Kjeldahl determination to give the diamino nitrogen. The remaining nitrogen is the monoamino nitrogen.

4. The filtrate and washings from the diamino determination were diluted to 500 cubic centimeters and an aliquot sample removed and the nitrogen therein determined by Kjeldahl. This gives the monoamino nitrogen.

5. Total nitrogen was determined on all of the samples by removing an aliquot portion and determining nitrogen by the Kjeldahl method.

6. The technique followed for the Kjeldahl determination in all cases was as follows: The sample was digested with 35 cc. of concentrated sulfuric acid, a small piece of copper, and about 8 grams of potassium sulfate being added to the digestion mixture. Digestion was continued for about four hours after the color of the solution cleared.

TABULAR DATA CONCERNING SAMPLES

TABLE I

WOOL

100 gm. Wool in two liters of hydrochloric acid.

Sample Number	Date	Conc. Acid	Biuret neg.	
	1925		1925	1926
1	Aug. 14	12N (approx.)	Dec. 1	
2	Aug. 14	10N	Dec. 16	
3	Aug. 13	7N	Dec. 23	
4	Aug. 13	6N		Feb. 2
5	Aug. 13	5N		Apr. 15
6	Aug. 13	4N		
7	Aug. 12	3N		
8	Aug. 12	2N		
9	Aug. 11	N (broken)		
10	Aug. 11	N/2		
11	Aug. 12	N/4		

CASEIN

100 gm. Casein in two liters of hydrochloric acid.

Sample Number	Date	Conc. Acid	Biuret neg.	
	1925		1925	1926
1	Nov. 21	12N (approx.)	Dec. 20	
2	Nov. 21	10N		Jan. 15
3	Nov. 21	9N		Feb. 18
4	Nov. 21	8N		Mar. 8
5	Nov. 21	7N		Apr. 18
6	Nov. 21	6N		
7	Nov. 21	5N		
8	Nov. 21	4N		
9	Nov. 21	3N		
10	Nov. 21	2N		
11	Nov. 21	1N		
12	Nov. 21	N/2		
13	Nov. 21	N/4		
14	Nov. 21	N/10		

TABLE II — *Tabular Account of Results*

WOOL

Wool, boiled with concentrated hydrochloric acid, eight hours, 100 gm. in two liters of solution. 20 cc. samples were analyzed.

Number	Amide Mgs.	Humin Mgs.	Diamino Mgs.	Monoamino Mgs.
1	8.6	5.0	29.7	96.0
2	8.6	7.2	29.5	98.0
3	8.7	6.1	31.8	96.0
4	8.6	....	28.3	98.0
Average	8.6	6.1	29.8	97.0
Percent of total Nitrogen	6.0	4.26	20.8	67.7

Total nitrogen recovered (sum of the above) ..... 142 Mgs.
Total nitrogen determined by Kjeldahl ..... 143 Mgs.
Percent of nitrogen recovered 99.2%

TABLE II — Continued

Sample No. 1 (p. 9). 100 gm. wool in two liters concentrated hydrochloric acid, stood from Aug. 14 to Dec. 1, 1925.

Number	Amide Mgs.	Humin Mgs.	Diamino Mgs.	Monoamino Mgs.
1	7.5	....	32.1	90.0
2	7.2	....	30.2	84.0
3	7.2	....	.....	84.0
4	7.4	4.5	27.3	.....
5	7.3	3.9	30.3	.....
6	7.5	4.8	29.4	86.0
Average	7.35	4.4	29.8	86.0
Percent of total Nitrogen	5.25	3.4	21.28	61.4

Total nitrogen recovered (sum of above) ..... 127.55 Mgs.
Total nitrogen determined by Kjeldahl ..... 140.0 Mgs.
Percent of nitrogen recovered 91.2%

TABLE II — Continued

WOOL

Sample No. 2 (p. 9). 100 gm. wool in two liters 10N hydrochloric acid stood from Aug. 14 to Dec. 16, 1925.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	6.2	....	24.5	95.0
2	....	4.5	.....	96.6
3	6.7	3.6	23.5	95.8
4	6.3	....	25.0	95.0
5	....	5.4	.....	95.5
6	6.1	5.2	24.9	95.5
Average	6.3	4.7	24.5	95.4
Percent of total Nitrogen	4.64	3.47	18.08	70.04

Total nitrogen recovered (sum of above) ..... 130.9 Mgs.
Total nitrogen determined by Kjoldahl ..... 135.8 Mgs.
Total of nitrogen recovered 96.5%

TABLE II — Continued

WOOL

Sample No. 3 (p. 9). 100 gm. wool in two liters 7N hydrochloric acid stood from Aug. 14 to Dec. 23, 1926.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	6.7	....	.....	85.0
2	6.9	....	33.1	.....
3	6.7	....	32.1	.....
4	6.9	4.0	36.8	84.0
5	6.4	4.9	32.8	84.0
6	....	4.3	34.7	.....
7	....	6.3	35.3	82.0
8	....	6.1	.....	86.0
9	....	5.3	.....	86.0
10	6.3	5.6	35.0	.....
Average	6.6	5.2	34.2	84.5
Percent of total Nitrogen	5.0	3.95	25.9	64.2

Nitrogen recovered (sum of above) ..... 130.5 Mgs.
Total ditrogen determined by Kjaldahl ..... 132.0 Mgs.
Percent of nitrogen recovered 98.9%

TABLE II — Continued

WOOL

Sample No. 4 (p. 9). 100 gm. wool in two liters of 6N hydrochloric acid, stood from Aug. 13, 1925, to Feb. 15, 1926.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	6.5	5.5	35.4	80.0
2	6.7	6.3	38.0	80.0
3	6.3	5.5	37.3	82.0
4	6.5	7.7	32.0	76.0
5	6.6	----	34.0	80.0
6	----	7.7	33.0	------
Average	6.52	6.54	34.9	79.6
Percent of total Nitrogen	4.87	4.87	26.0	59.4

Total nitrogen recovered (sum of above) ..... 127.6 Mgs.
Total nitrogen determined by Kjelhahl ..... 134 Mgs.
Percent of total nitrogen recovered 95.5%

TABLE II — Continued

WOOL

Sample No. 5 (p. 9). 100 gm. wool in two liters of 5N hydrochloric acid stood from Aug. 14, 1925, to April 15, 1926.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	6.3	4.5	24.0	92
2	6.3	4.7	24.8	86
3	7.4	6.9	26.0	90
4	5.5	----	32.0	86
5	6.0	4.8	29.7	86
6	6.1	5.3	28.5	88
Average	6.5	5.2	27.5	88
Percent of total Nitrogen	4.76	4.0	21.9	67.6

Total nitrogen recovered (sum of above) ..... 126.9 Mgs.
Total nitrogen determined by Kjeldahl ..... 130.0 Mgs.
Percent of total nitrogen recovered 97.6%

TABLE III

CASEIN

Casein, boiled with concentrated hydrochloric acid, 100 gm. in two liters of solution. 20 cc. samples analyzed.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	13.0	3.9	29.0	80.0
2	12.42	4.0	33.0	82.0
3	12.44	3.6	30.9	84.0
Average	12.6	3.8	30.9	82.0
Percent of total Nitrogen	9.45	2.83	23.0	61.0

Total nitrogen recovered (sum of above) ..... 129.3 Mgs.
Total nitrogen determined by Kjeldahl ..... 134.4 Mgs.
Percent of total nitrogen recovered 96.5%

TABLE III — Continued

CASEIN

Sample No. 1 (p. 9). 100 gm. casein in two liters concentrated hydrochloric acid, stood from Nov. 21, 1925, to Dec. 20, 1925.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	11.0	3.3	-----	-----
2	-----	8.8	-----	76.0
3	10.5	8.0	-----	78.0
4	10.0	----	-----	78.0
5	11.7	4.5	27.6	-----
6	10.9	8.8	-----	72.0
7	11.3	----	-----	76.0
8	10.5	----	-----	76.0
9	12.0	3.1	-----	-----
10	11.8	6.2	19.4	-----
11	-----	3.6	22.2	-----
12	10.1	4.7	20.0	86.0
13	-----	----	26.5	76.0
14	-----	7.1	34.8	74.0
15	-----	----	23.8	80.0
16	-----	6.9	23.4	-----
17	-----	7.8	24.5	-----
Average	11.0	5.83	26.9	77.2
Percent of total Nitrogen	8.26	43.7	20.2	58.0

Total nitrogen recovered (sum of above) ..... 120.93 Mgs.
Total nitrogen determined by Kjeldahl ..... 133.0 Mgs.
Percent of total nitrogen recovered 91.0%

TABLE III — Continued

CASEIN

Sample No. 2 (p. 9). 100 gm. casein in two liters 10N hydrochloric acid, stood from Nov. 21, 1925, to Jan. 25, 1926.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	10.7	----	27.0	78.0
2	10.9	6.9	28.1	74.0
3	11.5	4.4	31.1	74.0
4	12.0	4.0	30.2	76.0
5	12.0	6.6	------	74.0
6	12.1	4.0	------	74.0
Average	11.5	5.2	29.1	75.0
Percent of total Nitrogen	8.85	4.0	22.19	57.7

Total nitrogen recovered (sum of above) ..... 120.8 Mgs.
Total nitrogen determined by Kjeldahl ..... 130.0 Mgs.
Percent of total nitrogen recovered 93.0%

TABLE III — Continued

CASEIN

Sample No. 3 (p. 9). 100 gm. casein in two liters of 9N hydrochloric acid, stood from Nov. 24, 1925, to Feb. 18, 1926.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	11.1	6.4	29.4	------
2	11.2	8.2	29.4	72.0
3	10.9	6.0	------	------
4	11.5	5.4	30.0	74.0
5	12.0	5.0	------	72.0
6	------	5.7	28.5	------
7	------	----	27.5	------
Average	11.3	6.1	28.9	73.0
Percent of total Nitrogen	8.6	4.64	22.0	55.5

Total nitrogen recovered (sum of above) ..... 119.3 Mgs.
Total nitrogen determined by Kjeldahl ..... 131.5 Mgs.
Percent of total nitrogen recovered 91.0%

TABLE III — Continued

CASEIN

Sample No. 4 (p. 9). 100 gm. casein in two liters of 8N hydrochloric acid, stood from Nov. 21, 1925, to Mar. 8, 1926.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	12.4	5.0	36.0	70.0
2	10.2	4.0	36.6	72.0
3	11.4	6.7	33.6	70.0
4	12.0	....	27.1	67.4
5	10.1	....	.....	.....
6	11.5	....	.....	.....
7	11.5	4.3	23.8	62.0
8	11.3	6.4	27.3	62.0
9	10.4	5.2	27.7	.....
10	10.8	5.9	14.5	72.0
11	12.1	....	16.5	.....
Average	11.3	5.3	29.2	68.0
Percent of total Nitrogen	9.05	4.24	23.19	54.5

Total nitrogen recovered (sum of above) ..... 113.8 Mgs.
Total nitrogen determined by Kjeldahl ..... 125.0 Mgs.
Percent of total nitrogen recovered 91.0%

TABLE III — Continued

CASEIN

Sample No. 5 (p. 9). 100 gm. casein in two liters of 7N hydrochloric acid, stood from Nov. 24, 1925, to April 18, 1926.

Number	Amide	Humin	Diamino	Monoamino
	Mgs.	Mgs.	Mgs.	Mgs.
1	11.6	6.5	31.3	74.0
2	11.6	6.5	.....	70.0
3	12 2	7.7	32.6	70.0
4	12.1	....	30.4	64.0
5	12.1	7.9	31.5	.....
Average	11.9	7.1	31.9	69.0
Percent of total Nitrogen	9.35	5 57	25.0	54.2

Total nitrogen recovered (sum of above) ..... 119.9 Mgs.
Total nitrogen determined by Kjeldahl ..... 127.3 Mgs.
Percent of total nitrogen recovered 94.2%

Table IV

RECAPITULATION OF RESULTS (WOOL)

Sample	Amide		Humin		Diamino		Monoamino		Total Cd	Total Fd	Recd
Boiled	Mgs.	%	Mgs.	%	Mgs.	%	Mgs.	%	Mgs.	Mgs.	%
Boiled	8.6	6 0	6.1	4.26	29.8	20.8	97.0	67.7	142.0	143.3	99.2
1	7.35	5.25	4.4	3.4	29.8	21.28	86.0	61.4	125.55	140.0	91.2
2	6.3	4.64	4.7	3.47	24.5	18.1	95.4	70.04	130.9	135.8	96.5
3	6.6	5.0	5.2	3.9	34.2	25.9	84.5	64.0	130.5	132.0	98.9
4	6.52	4.87	6.54	4.87	34.9	26 0	79.6	59.4	127.6	134.0	95.3
5	6.2	4.76	5.2	4.0	27.5	21.9	88.0	67.6	126.9	130.0	97.6

RECAPITULATION OF RESULTS (CASEIN)

Boiled	12.6	9 45	3.8	2.83	30.9	23.0	82.0	61.7	129.0	134.4	96.5
1	11.0	8.26	5.8	4.37	26.9	20.2	77.2	58.0	120.9	133.0	91.0
2	11.5	8.85	5.2	4.0	29.1	22.19	75.0	57.7	120.8	130.0	93.0
3	11.3	8.6	6.1	4.64	28.9	22.0	73.0	55.5	119.3	131.5	90 9
4	11.3	9.05	5.3	4.24	29.2	23.19	68.0	54.5	113.8	125.0	91.0
5	11.9	9.35	7.1	5.57	31.9	25.0	69.0	54.2	119.9	127.3	94.2

DISCUSSION OF RESULTS

In the examination of the figures several interesting factors may be noted. The least deviation of quantities of nitrogen determined within any of the samples is 0.1 mg., occurring in the determination of amide nitrogen in the sample of wool boiled under reflux. The greatest variation occurs, as might be expected, in the monoamino determination (that of Sample No. 1 of casein where a range of 14 mgs. is noted). The largest source of error seems to be likely to occur in the monoamino determination, as it is made upon the aliquot part of an aliquot part. Consequently, the errors might be subject to multiplication. However, the error of 14 mgs. is of the order of 1.4 per cent of the original sample. In the recovery of nitrogen the results are much less satisfactory than was expected. The lowest per cent of nitrogen recovered was 90.9 per cent in the case of Sample No. 3 of casein; the greatest per cent of nitrogen recovery was 99.2 per cent in the boiled sample of wool.

In view of the fact that the determinations of the monoamino and diamino values are made from the filtrate and washings from the humin determinations, it might be concluded that the insufficient washing of the humin precipitate would lead naturally, to an increase in humin value, with a corresponding decrease in diamino and monoamino values. A careful examination of the data does not reveal such a relationship. Hence the conclusion that the five-fold washing of the humin precipitate as noted in the experimental details of the work is sufficient to remove the monoamino and diamino acids from the humin precipitate. However, if there is occlusion of nitrogen compounds in the finely divided precipitate, the above argument would fail to hold. However, it seems reasonable to assume that the error would be practically constant, because in so far as possible uniform conditions are maintained.

A similar examination of the data leads to the conclusion that the removal of the amide nitrogen is complete, since if the distillation were insufficient to remove all the ammonia from the alkalinized reaction mixture, the excess nitrogen would show up in the mono- and diamino groups, which is not indicated by the figures. (From the nature of the method, there would be no change in the humin value.)

A check upon completeness of ammonia removal was carried out as follows: The regular operation was carried out,

and the reaction flask again diluted with ammonia-free distilled water and a second vacuo distillation carried out. No increase in alkalinity of the solution in the receiver was observed. This check was made on several samples during the early part of the experimental work.

Completeness of the precipitation of the diamino groups was checked by testing the filtrate from the regular procedure with the addition of excess of phosphotungstic acid reagent. No additional precipitation was noted, and the findings of Osborne and Harris, loc. cit., in regard to the quantity of the precipitant required were found to be applicable to our work.

The monoamino values were determined by loss as well as by actual analysis. This method, of course, would indicate 100 per cent recovery of nitrogen. The percentage of monoamino nitrogen as determined by loss is in every case higher than the percentage as determined by actual analysis. This indicates that the largest error occurs in the monoamino value.

The work of Blish and Gortner [16] indicates that the formation of humin nitrogen is due to the decomposition of tryptophane (which occurs in the monoamino group). If this is the case we should expect to note an increase in the monoamino values with a decrease in humin values. The data show such a relationship. Particularly in the values for casein, which is relatively higher in tryptophane content than in wool, there is a fairly regular increase in humin value with a corresponding decrease in monoamino values, as the concentration of the hydrochloric acid was lowered.

The work of Fischer and Abderhalden,[17] et. seq. on the products of partial hydrolysis of proteins leads to the conclusion that polypeptides are formed, containing two, three, four and even greater number of amino acids linked together in their molecules. They have shown that many of these are soluble and are precipitated as their phosphotungstates. This would indicate that if these occur in the lower concentrations of acid that an increase in the diamino nitrogen value would be noted. In this work larger diamino values are, however, in the lower acid concentrations than in the higher concentrations. Whether this fact is due to the formation of the phosphotungstates of polypeptides or not, cannot be definitely stated. However, it is hoped that work

[16] J.A.C.S. 37, 1630, (1915).
[17] Zeit. physiol. chem. 39, 2315, (1906).

soon to be started will lead to the isolation and identification of some of these products.

Polypeptides are known to form stable hydrochlorides which are insoluble. It is possible that the insoluble material found in increasing quantities as the concentration of hydrochloric acid is decreased is in part, at least, composed of insoluble hydrochlorides of some of the polypeptides. Some of these products are known also to give the biuret test, but in the present case, the insoluble residue give a negative biuret, however this not a criterion for the absence of polypeptides.

In conclusion it may be stated that work is in progress at this laboratory which it is hoped will lead to the isolation and identification of some of the products of the partial hydrolysis of protein material. The present research represents the preliminary work of a series of studies of proteins hydrolysed at room temperature.

SUMMARY

1. A detailed discussion of Hausmann's method, as modified by Osborne and Harris, for the determination of the distribution of nitrogen in the hydrolysates of protein material is given, together with data regarding its applicability to the hydrolysates of wool and casein.

2. Some minor alterations in the above methods have been tested and found applicable.

3. A study has been made of effect of various concentrations of hydrochloric acid on the nitrogen distribution in hydrolysates of wool and casein.

4. There is a fairly regular increase in the humin nitrogen value with a somewhat corresponding decrease in the quantity of monoamino nitrogen, as the concentration of the acid is lowered.

5. A possible explanation of certain relationships found to exist in the values is presented.